

ANALYSIS OF POTATO SUGARS
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ABSTRACT

A procedure for determining sugars in potato tubers by gas chromatography is described and examples given of its application to stored and reconditioned potatoes. Juice from tubers is extracted, boiled, centrifuged, and the trimethylsilyl derivatives of the sugars prepared. Column choice and operating conditions are given. Results are fairly comparable to those from wet analysis, are more comprehensive, and more easily obtained.

RESUMEN

Se describe un procedimiento para determinar azúcares en tubérculos de papa por medio de gas-cromatografía y se dan ejemplos de su aplicación a papas almacenadas y restoradas. El jugo de los tuérculos se extrae, cuece, y centrifuga y los derivados trimethylsilyl de azúcar se preparan. Se indica la selección de la columna y las condiciones de operación. Los resultados se comparan bien a los obtenidos por un análisis húmedo, son más comprensivos y se obtienen más fácilmente.

In the course of our study of carbohydrate transformation in potatoes, it became necessary to analyze for the individual sugars such as sucrose, glucose, and fructose. The standard chemical methods for this purpose are cumbersome, particularly in view of the complex mixture in tuber extracts. Gas chromatography for measurement of carbohydrates has been an extensively used technique since 1960. One of the problems in devising a gas chromatographic technique for a specific carbohydrate purpose is to review the over 150 publications on the subject. We will not try to cover the literature here, but refer to three reviews: Wells, et al (12), Sloneker (9), Herb (6).

In considering the use of gas chromatography for the analysis of potato sugars, four problems were evident; carbohydrate extraction methods, derivatization and solvents, types of column packing, and quantitation of the procedure. It is the purpose of this paper to report on our evaluation of alternate solutions to these problems; to recommend a specific technique and to demonstrate its utility.

MATERIALS AND METHODS

Extracts of the tubers were prepared by three methods: (i) classical 80% alcohol extract, (3) (ii) boiled centrifuged juice, wherein tubers

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TABLE 1.—*Characteristics of columns evaluated.*

Type		Temperature limits	Amount	Support	Size
Non-Polar	OV-1 (Methyl silicone)	350 F	3%	Gas Chrom Q 60/80 mesh	6'x¼" S.S.
Polar	ECNSS-M (30% Cyanothyl- methyl silicone in ethylene succinate chain)	200 F	3%	Gas Chrom Q 100/120 mesh	7'x⅛" S.S.
Intermediate polarity	Ethylene glycol succinate	180 F	0.2%	Gas Chrom P. 100/120 mesh	4'x⅛" S.S.
	Ethylene glycol adipate SF 1150 Silicone 50		0.2% 0.4%		
Polar (Special treated)	Carbowax 20M terminated with terephthalic acid	190 F	10%	Gas Chrom W HMDS treated 80/100 mesh	7'x⅛" S.S.
Selective	OV-17 Methyl silicone (50% phenyl substituted)	320 F	3%	Gas Chrom Q 60/80 mesh	6'x¼" Copper

were ground through a Juicerator⁴ and allowed to settle for a few minutes, then about 10 ml was heated in boiling water 3-5 min to inactivate enzymes and precipitate proteins and clarified by centrifugations, (iii) raw, centrifuged juice, wherein juice is only centrifuged (5,000 xg for 15 min.) to remove starch and cell debris. Aliquots (0.1 ml) were placed in 4 ml vials (polyethylene stoppers) and were dried at room temperature with a jet of nitrogen.

Derivatives (TMS, *o*-trimethylsilyl ethers) were prepared by adding 0.5 ml of reagent mixture containing pyridine: hexamethyldisilane: trimethylchlorosilane in ratio of 10:2:1. If the sugar concentration was expected to be over 5% total sugars, then 1.0 ml of reagent mixture was added. Preliminary tests indicated a minimum of 2 hours was required for complete derivatization and most samples were reacted overnight. (Other derivatives (DMS, etc.) reported in literature did not show promise.)

Columns and their characteristics are listed in Table 1.

When solvents such as methylene chloride, hexane, or chloroform were tried in place of pyridine, the derivatives were made in the usual way with TMS reagent mixture, the excess reagents were removed by a jet of nitrogen and the TMS derivative was taken up in an equivalent amount of the alternate solvent. All solvents were carefully dried prior to use. Residual NH₄Cl (from the TMS reaction) did not interfere.

⁴Mention of company or trade names does not imply endorsement by the Department of Agriculture over others not named.

Individual peaks on the chromatogram were identified by comparison with derivatives of authentic sugars on at least two columns. Derivatives of authentic sugars were made by dissolving 0.100 gm in 10 ml of water, allowed to stand overnight to equilibrate any possible isomers, then 0.1 ml was dried and derivatized as with the extract samples. Ion exchange and paper chromatographic methods were employed to verify the identity of some of the sugars.

Quantitation was obtained by use of a standard curve since the multiplicity of peaks made the use of an internal standard impractical. This was obtained by dissolving 0.500 g. each of fructose, glucose, and sucrose in 50 ml. of deionized water. After evaporation of a 0.1 ml aliquot, 1.0 ml of TMS reagent was added. Thus, 1.0 μ l contained 1.0 μ gm of each sugar.

For analysis, 1.0 - 3.0 μ l were injected into the column and the instrument settings were selected to give a reasonable deflection on the recorder.

The gas chromatograph was a late model instrument equipped with dual columns, differential hydrogen flame detector and temperature programming. The recorder was matched to output of gas chromatograph (5 mv) and equipped with disc integrator.

RESULTS AND DISCUSSION

Preparation of Extracts. The raw juice gave erratic chromatograms, mostly non-reproducible and with a certain amount of non-symmetry in the peaks. The dried protein material appeared to interfere in the solubilization of the carbohydrates in pyridine, which is a necessary first step in making the TMS derivatives. Wells (12) reported a similar problem with biological fluids and found it necessary to deproteinize before attempting the derivatization.

Carbohydrate extraction by the alcohol technique involving several mass extractions with cold alcohol followed by extraction with boiling alcohol gave chromatographic results similar to those obtained chemically. However, when the chromatogram from the alcohol extracts was compared with that from the boiled centrifuged juice, two definite differences were noted. A major peak (peak 1) in the hexose region of the chromatogram was significantly smaller in the alcohol extract than in the boiled juice as indicated in Fig. 1. Furthermore, a small peak (about 20% of the sucrose peak) appeared in the di- and trisaccharide region as seen in Fig. 2. It was assumed that these changes were due to the prolonged heating in alcohol, although differences in solubility in 80% alcohol versus water may account for these changes.

Boiled centrifuged juice appears to be adequate for the study of sugar transformation in potato tubers.

Derivatives. Derivatives other than TMS have been employed for the gas chromatographic analysis of carbohydrates. For example, the DMS (*O*-dimethylsilyl ethers) were recommended for oligosaccharides (9), but such derivatives are unstable in the presence of trace quantities of water and in the presence of excess reagents other complexes are formed. The alditol acetates (*O*-acetyl esters) (9) yield only one derivative for any particular sugar, thus the difference between α - and β -glucose could not

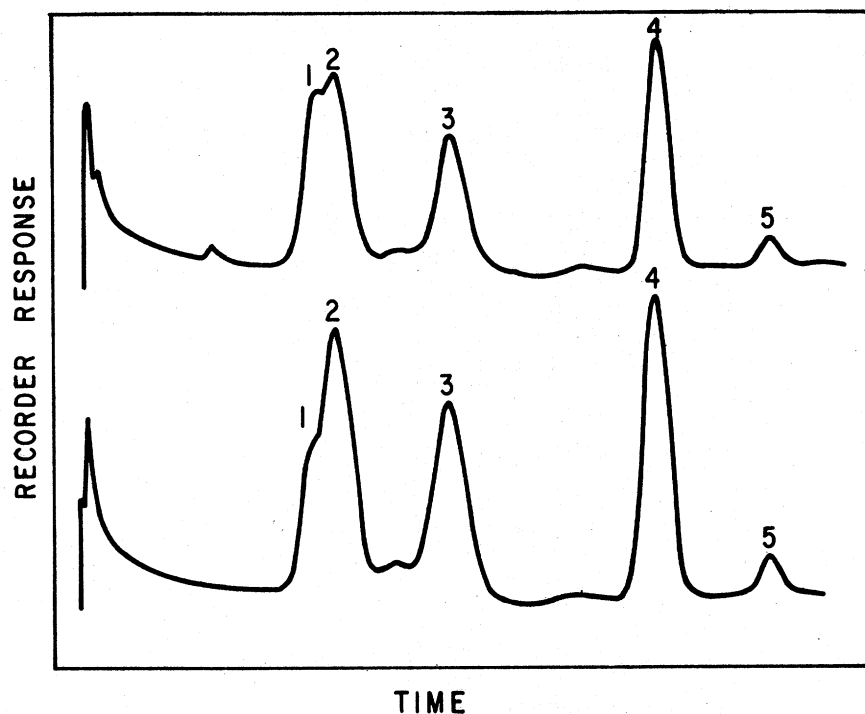


FIG. 1.—TMS derivatives from boiled centrifuged juice (upper curve) and alcohol extract of tuber (lower curve) Col. OV-1, 185°C. Peaks: 1-unknown, 2-fructose, 3- α -glucose, 4- β -glucose, 5-inositol.

be shown. Also, fructose and glucose produce the identical alditol derivative. *Methyl ethers* are fairly difficult to prepare. *TFA* (*O*-trifluoroacetyl ester) is a fairly recent development (9) primarily for use with oligosaccharides and the properties of this derivative are still being studied. The *TMS* (*O*-trimethylsilyl ethers) derivatives are stable and the method has been well developed into a quantitative, rapid procedure as indicated by Sweeley (10). The necessary reagents are readily available and usually premixed in the optimum ratios. The fact that this method yields specific derivatives for all configurations of each sugar may actually be advantageous. For these reasons, TMS derivatives were selected for this work.

Column Characteristics. The *non-polar*, OV-1 column produced too much pyridine tailing to permit quantitation of pentose sugars and smaller molecules. However, the separation of mono-, di-, and trisaccharides were excellent under optimum conditions. Temperature programming characteristics were excellent and very little tailing resulted with methylene chloride or hexane. Chloroform could not be employed with the silicone materials because of excessive bleeding. The *polar*, ECNSS-M column also produced excessive pyridine tailing but somewhat less tailing was obtained with methylene chloride and hexane. The peaks were well defined but resolution was not greatly better than with OV-1. The *inter-*

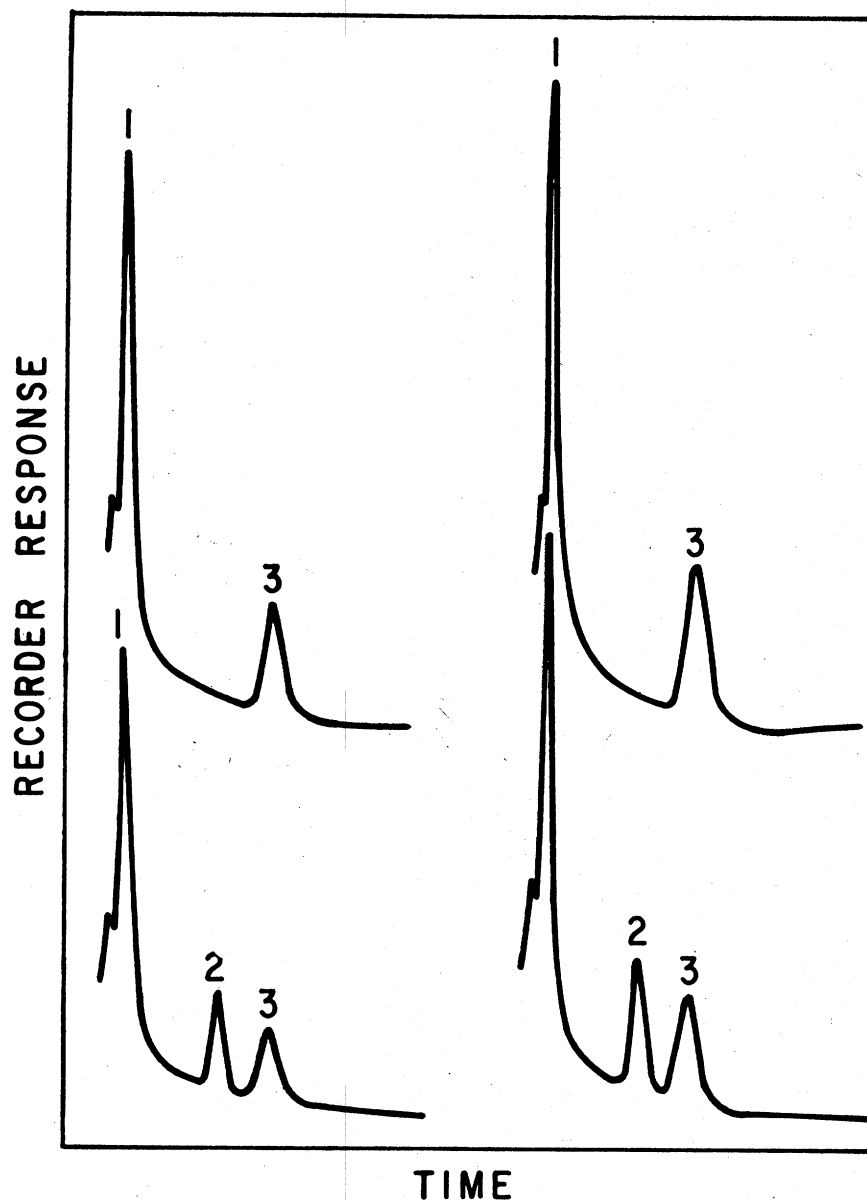


FIG. 2.—TMS derivatives from boiled centrifuged juice, two samples, (upper curves) and alcohol extract of tuber, two samples, (lower curves) Col. OV-1 280°C. Peaks: 1-sucrose, 2-additional peak in alcohol extract, 3-unidentified oligosaccharide.

mediate polarity column (Albersheim, 1) produced excessive tailing with pyridine but almost none with methylene chloride (Fig. 3) or hexane. Resolution of the sugars was not obviously better than with OV-1.

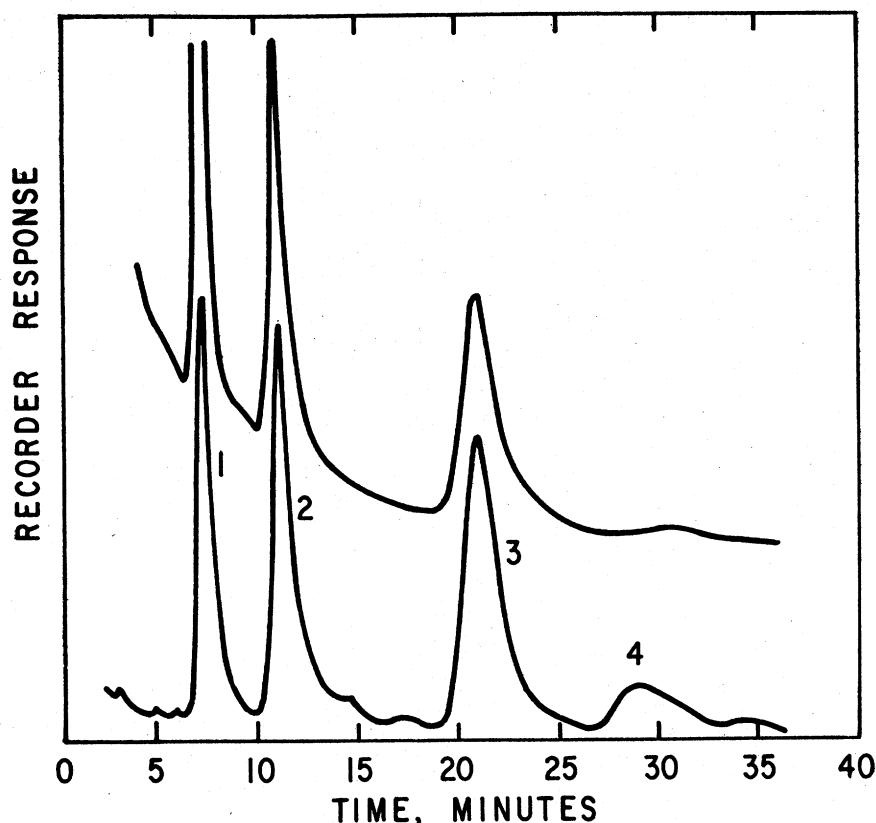


FIG. 3.—Methylene chloride (lower curve) as alternate solvent for pyridine (upper curve) Col. Intermediate Polarity, 170 C. Peaks: 1-fructose, 2- α -glucose, 3- β -glucose, 4-inositol.

The *polar, specially treated*, Carbowax-20M produced excessive tailing with pyridine but very little with the methylene chloride or hexane solvents. Of all the columns tried, this was the only one that separated β -fructopy ranose from β -fructofuranose. An aqueous solution of fructose contains about 15% of the furanose form at equilibrium, as indicated by Verstraeten (11), and Fig. 4 clearly shows this fact. This column may be employed for special purposes. The *selective*, OV-17 column was very similar in performance to the OV-1 column. It does show slightly different retention times (relative to α -glucose) than OV-1 and it may have significantly different relative times for sugar acids and sugar phosphates. With these observations in mind, columns OV-1 and OV-17 were selected for further study and the Carbowax column was used for special purposes.

Retention times of monosaccharides, relative to α -glucose, and of di- and trisaccharides, relative to sucrose are shown in Table 2. Although these values were determined in an extremely careful manner and are quite accurate for these columns and operating conditions, they should be used only as a guide. Each packed column may be greatly different from

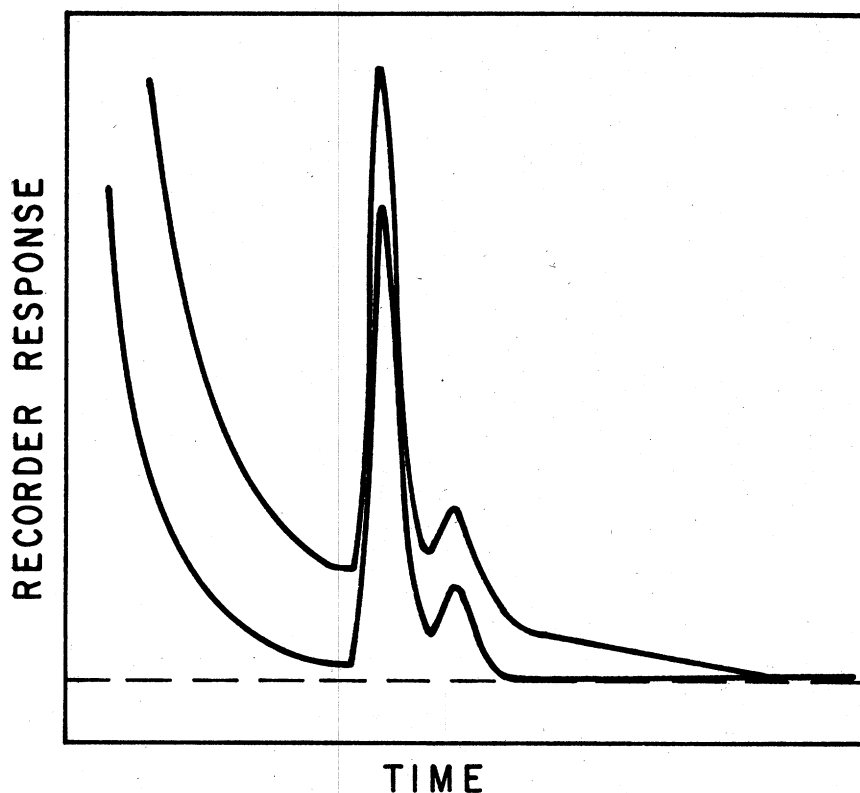


FIG. 4.—Pyranose (large peak) and furanose (small peak) forms of β -fructose. Upper curve with pyridine, lower curve with methylene chloride solvent. Col. Carbowax 20M, 135 C.

another column similarly prepared and, thus, the retention times should be determined for each individual column. It was noted that any carbohydrate derivative requiring more than 40 min. for elution had a tendency to smear and was difficult to quantitate. Additionally, the first derivative to elute should appear after the solvent peak. The reference material, α -glucose, was selected because it can be obtained in high purity, is always present in potatoes and always exists as an equilibrium of anomers in aqueous solution. Furthermore, the ratio of α/β glucose is 40/60 (10) and, therefore, fixes two reference points. Since the ratio of α/β glucose changes with time after derivative preparation, this ratio is only valid for freshly prepared materials. Sucrose was similarly selected although only a single peak is obtained.

The final procedure selected is to take 0.1 ml boiled, centrifuged juice, dry, add 0.5 ml TMS reagents and let stand at least two hours. Chromatography on columns OV-1 and/or OV-17 at 180 C and 260 C. Compare with the standard chromatogram involving known amounts of glucose, fructose, and sucrose.

Different parts of a sugar chromatogram can be emphasized by altering the temperatures of the column. For example, Curve c in Fig. 5

TABLE 2.—Retention time relative to α -glucose for monosaccharides and relative to sucrose for di- and trisaccharides.

Column type	Carbowax		OV-1			OV-17		
	135 C	190 C	170 C	185 C	280 C	170 C	185 C	280 C
Temperature								
α -Arabinose	0.30		0.30	0.32		0.47	0.35	
Ribose	.35		.35	.35		.50	.43	
β -Arabinose	.45		.32	.35		.52	.40	
α -Xylose	.55		.45	.47*		.65	.60*	
Fructofuranose	.55		.75*	.76*		.60*	.65*	
α -Mannose	.60		.72	.72		.73	.66	
Fructopyranose	.65							
β -Xylose	.85		.55			.72		
α -Galactose	.90		.90	.90		.85	.82	
α -Glucose	1.00		1.00	1.00		1.00	1.00	
β -Mannose	1.12		1.11	1.07		.95	.93	
β -Galactose	1.30		1.07	1.07		.97	1.00	
β -Glucose	2.05		1.60	1.50		1.23	1.42	
α -Galacturonic Acid			1.45	1.27		1.42	1.67	
α -Gluconic Acid			1.47	1.35			1.77	
β -Galacturonic Acid			1.72	1.65		1.70	1.97	
β -Gluconic Acid			1.90	1.75			1.92	
Mannitol				1.25			.90	
Inositol				2.3			1.80	
Sucrose		1.0			1.0			1.0
α -Maltose		1.3			1.1*			1.1*
β -Maltose		1.6						
α -Melibiose		3.0			1.6*			1.7*
β -Melibiose		3.6						
Gentiobiose					1.8*			1.9*
C ₁₈ -1					2.3			
C ₁₈ -2					3.2			
Raffinose					6.3			5.8

*Single peak for both forms of the sugar indicated.

emphasizes the disaccharides while Curve a emphasizes the trisaccharides. In normal runs, the monosaccharides were studied first and then the disaccharides. By using temperature programming, the entire range of the sugars can be studied as shown in Fig. 6. Some loss in precision results from this technique.

Fig. 7 is typical of hexose sections of chromatograms and changes in fructose and β -glucose are easily followed. α -glucose is two-thirds of β -glucose, and when this value is subtracted from peak 5, changes can be followed in an unknown component (retained on an anion exchange resin in column chromatography; the same "unknown" component as peak 1 in both Fig. 1 and Fig. 6). Apparently, inositol (peak 7) does not significantly change with reconditioning of Kennebecs.

If one knows the dry matter content in the potatoes under investigation, then a simple calculation can give results similar to results from traditional official methods of analysis of AOAC. Table 3 compares gas chromatographic analysis for fructose, glucose, and sucrose in juice and alcoholic extract with wet analysis of the same extracts. Most of the differences in the values can be accounted for by the insoluble starch and other substances that are replaced by alcohol in the alcohol extract

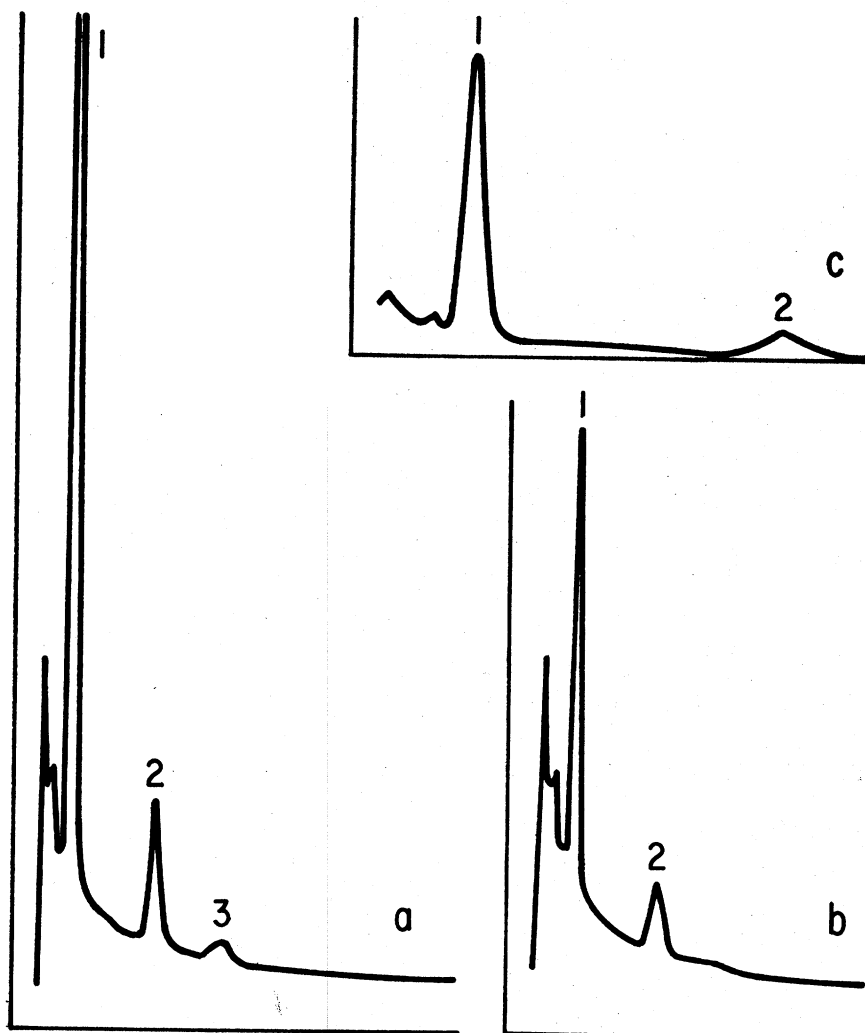


FIG. 5.—Focusing on TMS derivatives of di- and oligosaccharides. Col. OV-17 (a) Chromatographed at 290 C, boiled centrifuged juice from cold storage Kennebecs. (b) Chromatographed at 290 C, boiled centrifuged juice from Kennebec reconditioned 8 weeks at 18 C. (c) Chromatographed at 260 C, same as curve (a). Peaks: 1-sucrose, 2-unidentified oligosaccharide, 3-unidentified oligosaccharide.

procedure. (The Kennebec potatoes used in this test had a specific gravity of 1.103 or about 19% starch). The values for wet analysis are similar to those obtained by gas chromatography as one would expect if the principal sugars measured in both cases are fructose, glucose, and sucrose. But, because wet analysis involves additional potato components, a direct comparison would be meaningless.

A simple procedure to measure individual sugars being transformed

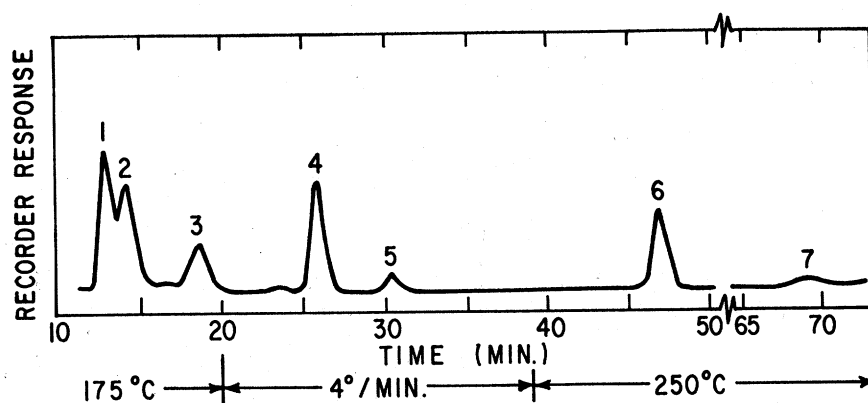


FIG. 6.—Temperature programmed run of TMS derivatives of boiled centrifuged juice. Col. OV-1 175 C initial temperature. Peaks: 1-unknown, 2-fructose, 3- α -glucose, 4- β -glucose, 5-inositol, 6-sucrose, 7-unidentified oligosaccharide.

TABLE 3.—Analysis of tuber extracts by two methods.¹

GAS CHROMATOGRAPHY						WET ANALYSIS					
Juice (%)			Alcohol Extract (% wet tuber)			Juice (%)			Alcohol Extract (% wet tuber)		
Fru	Glu	Suc	Fru	Glu	Suc	RS ³	Suc ⁴	TS ⁵	RS ³	Suc ⁴	TS ⁵
Kennebecs, Stored in cold ²											
	.37	.45	.25	.28	.36	.22	.80	.19	1.11	.60	.17 .73
Kennebecs Reconditioned ⁶											
	.05	.10	.16	.04	.06	.11	.19	.09	.40	.13	.10 .22

¹Each value is an average of three determinations. Standard Deviation for GLC values — 0.0094.

²Harvested 9/26/67, stored at 40 F to 6/17/68.

³Reducing sugars by Somogyi method, modified by Nelson (2).

⁴Sucrose by increase in reducing sugars after mild acid hydrolysis.

⁵Total sugars by anthrone method (2).

⁶Harvested 9/25/67, stored at 40 F to 5/8/68, held at 65 F to 6/17/68.

in potato tubers has been described which should be an aid for further studies; for example:

Schwimmer, Benevue, Weston, and Potter (8) used paper chromatography to study the sugars in deionized alcohol extracts of potatoes. Besides sucrose, the higher sugars they tentatively identified were stachyose, raffinose, melizitose, and melibiose, and fructans. We did not look for stachyose or fructans; we did not observe melibiose, melizitose, or maltose; we did observe a trace amount of what may be raffinose. Perhaps, if the potato juice were concentrated, these sugars could be raised to levels needed for positive identification. But we clearly did see two peaks (Fig. 5) in curve a which could easily be confused with one of the above oligosaccharides in paper chromatography.

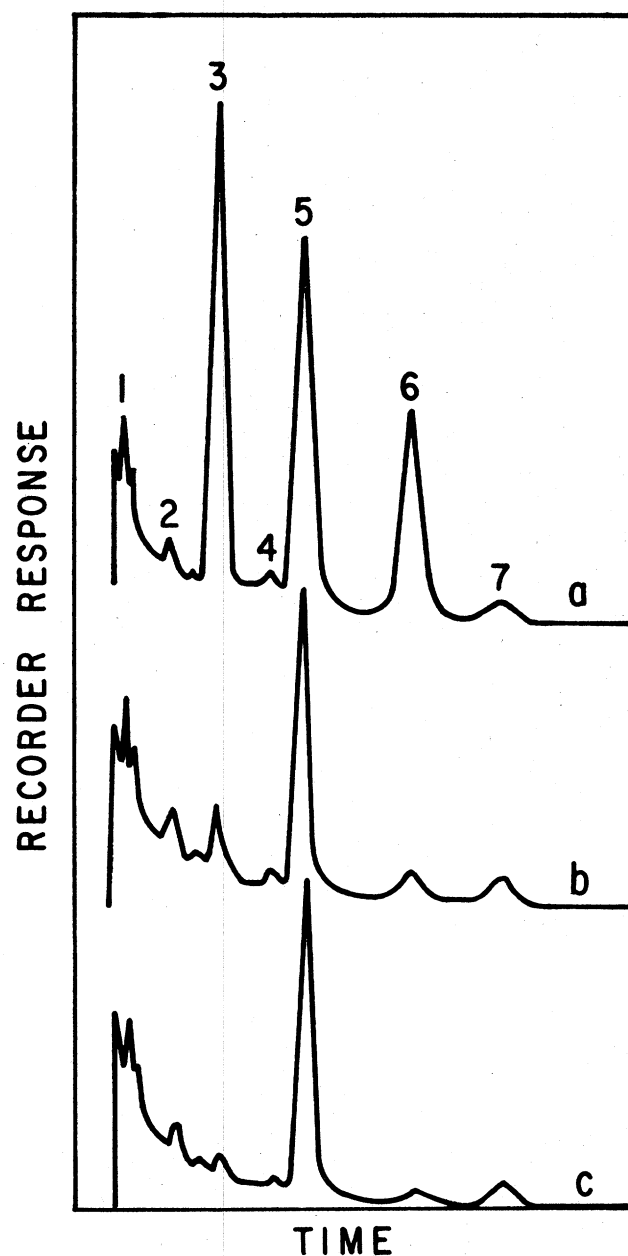


FIG. 7.—Changes in hexose section of chromatograms with reconditioning. TMS derivatives of boiled juice of Kennebecs. Col. OV-17, 185 C. Curve (a) out of 40 F storage; (b) reconditioned 4 weeks at 65 F; (c) reconditioned 8 weeks at 65 F. Peaks: 1-amino acid, 2-inorganic PO_4 , 3-fructose, 4-unknown, 5- α -glucose + unknown, 6- β -glucose, 7-inositol.

Because of greater response of fructose to temperature changes, Schwimmer et al (8) assigned an important role to fructose in storage changes. This may very well be true and now at least we can precisely study such changes.

Samotus and Schwimmer (7) reported that immature potatoes accumulate an excess of fructose over glucose during cold storage. This immediately suggests that a specific test to establish ratio of fructose/glucose after one month cold storage would identify immature potatoes. It is well known that immature potatoes do not recondition well, thus, this test would predict possible later troubles.

The significance of inositol is not known. It would be interesting to determine if inositol and its phosphorylated brother, phytic acid, are involved in carbohydrate transformation. Other phosphorylated carbohydrates (4, 5) have been determined as TMS derivatives, so presumably, phytic acid could be also.

It may be possible that there is a "normal" sugar distribution, as shown by GC, for each specific variety grown in good health, and stored under optimum conditions. A deviation in ratios of important sugar components would indicate an abnormality, a potential problem in utilization.

By being able to work with a small amount of juice, the pattern of translocated carbohydrates could be determined by taking a sample of juice as it flows through the stolon from haulm to tuber.

In this work, it was assumed that the equilibrium between the various configurations of the sugars existed in the tuber prior to extraction of juice. However, in the case of fructose, equilibrium between the furanose and pyranose forms may take many hours at the low temperatures used in cold storage of potatoes (11). If we consider that furanose is the important configuration in carbohydrate transformation and that the furanose level is only 15% of total fructose, then the equilibrium rate, pyranose to furanose, may possibly affect rate of reaction involving fructose. Perhaps of more importance is the fact that the effective concentration of fructose for transformation purposes is only 15% of total fructose, whereas the reducing power and browning potential is equal to total fructose. Is, in fact, the low level of β -fructofuranose affecting sugar transformation? This and other questions are under study.

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